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Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 7: A one-month, randomized, ambulatory, controlled clinical study in Poland

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ABSTRACT

This randomized, open-label, ambulatory, controlled clinical study investigated biomarkers associated with cardiovascular risk and biomarkers of exposure to 10 selected harmful and potentially harmful constituents (HPHC) in cigarette smoke in 316 male and female Polish smokers. Subjects were randomized to continue smoking conventional cigarettes (CC; N = 79) or switch to smoking the Electrically Heated Cigarette Smoking System series-K cigarette (EHCSS-K6; N = 237). Biomarker assessments were performed at several time points during the study at baseline and during the 1-month investigational period. The primary biomarkers were high-sensitivity C-reactive protein and white blood cell counts. No statistically significant differences in the two primary biomarkers were found between the study groups at the end of the study. End-of-study comparisons of secondary biomarkers between study groups indicated an increase in high-density lipoprotein cholesterol, and reductions in red blood cell count, hemoglobin, and hematocrit levels in the EHCSS-K6 group. All biomarkers of exposure to cigarette smoke HPHC were decreased in the EHCSS-K6 group, despite an increase in cigarette consumption, compared to the CC group. There were no apparent differences in any of the safety assessment parameters between the groups, and the overall incidence of study-related adverse events was low.

1. Introduction

There is overwhelming medical and scientific consensus that cigarette smoking causes lung cancer, heart disease, emphysema, and other serious diseases in smokers (US Department of Health and Human Services, 2010). The Family Smoking Prevention and Tobacco Control Act (FSPTCA) (Family Smoking Prevention and Tobacco Control Act, 2009) in the United States has empowered the Food and Drug Administration (FDA) to evaluate and regulate modified risk tobacco products (MRTPs) (Deyton et al., 2010). The FDA, in consultation with the Institute of Medicine (IOM), has also been charged to issue guidance and regulations on the scientific evidence required for the assessment and ongoing review of MRTPs (Food and Drug Administration, 2012; Institute of Medicine, 2012).

'The association between cigarette smoking and cardiovascular disease (CVD) is well documented. For example, smoking is a cause of premature atherosclerosis, acute myocardial infarction, sudden death, and stroke (Burns, 2003; Hatsukami et al., 2006a,b). Significant changes in biomarkers related to oxidative stress, endothelial damage, thrombosis, inflammation, and lipid metabolism have been found in smokers (Benowitz, 2003; Hatsukami et al., 2006a,b; US Department of Health and Human Services, 2010).

Use of the Electrically Heated Cigarette Smoking System (EHCSS) series-K heater and the EHCSS series-K6 cigarette results in reduced levels of a wide range of toxicologically important cigarette smoke HPHC and significantly lowers the biological activity of mainstream smoke compared to conventional lit-end cigarettes in laboratory-based test systems (Werley et al., 2008; Zenzen et al., 2012). By heating tobacco, the temperature reached is lower than that reached in the burning cone of a conventional lit-end cigarette. Up to 8 puffs can be obtained by smoking an EHCSS series-K cigarette with the EHCSS heater. Previous clinical investigations with the EHCSS have found that there are reductions in biomarkers of exposure to selected cigarette smoke HPHC (Frost-Pineda et al., 2008a,b; Roethig et al., 2005, 2007). Smokers who switched to an earlier version of the EHCSS over 12 months also had favorable changes in several biomarkers associated with CVD (Roethig et al., 2008). Additionally, the use of an EHCSS results in reductions of the levels of environmental tobacco smoke (ETS) constituents in indoor air (Frost-Pineda et al., 2008c; Tricker et al., 2009).

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The current study investigated the effect of switching to the EHCSS series-K heater and the EHCSS-K6 cigarette for 1 month on the levels of biomarkers associated with CVD and selected biomarkers of exposure to cigarette smoke HPHC in smokers. The biomarkers associated with CVD included those (white blood cell count, hematocrit, urine 11-dehydrothromboxane B_2, and high-density lipoprotein cholesterol) for which statistically significant changes were observed in a previous 12-month study evaluating a second-generation EHCSS (series-JLI) (Roethig et al., 2008). Biomarkers of exposure were selected for the HPHC 1,3-butadiene, 2-naphthylamine (2-NA), 4-aminobiphenyl (4-ABP), acrolein, benzene, carbon monoxide (CO), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), nicotine, pyrene, and *o*-toluidine.

The primary biomarkers investigated were white blood cell (WBC) count and high-sensitivity C-reactive protein (*hs*-CRP), two biomarkers which indicate an inflammatory response associated with CVD and have been shown to be influenced by smoking (Abel et al., 2005; Andrews and Tingen, 2006; Bakhru and Erlinger, 2005; Bazzano et al., 2003; Hatsukami et al., 2005).

This is the final manuscript in a series of five clinical evaluations of the EHCSS describing data from clinical investigations performed under both controlled (Tricker et al., 2012a,b,c,d) and actual use smoking conditions (this study).

2. Materials and methods

2.1. Subjects

Subjects were eligible for enrollment if they were Caucasian smokers aged 30–60 years with acceptable health conditions. Subjects were current smokers of commercially available, non-mentholated conventional cigarettes with a 3–10 mg tar yield with a smoking history of at least 10 years prior to screening.

Subjects with clinically relevant abnormal findings based on the screening assessments were excluded. Pregnant or lactating women and women of child-bearing potential who were not using an acceptable method of contraception were also excluded. Subjects were recruited from the clinical site database and were compensated for their participation in the study. All subjects provided written informed consent and were advised that they were free to withdraw from the study at any time. Each subject was provided with advice and information about the risks of smoking, and counseling was made available.

2.2. Study design and conduct

This study was approved by the Independent Ethics Committee of the Regional Chamber of Physicians, Warsaw, Poland, and conducted at MTZ Clinical Research Ltd., Warsaw, Poland (MTZ) in compliance with the ethical principles that have their origin in the Declaration of Helsinki (World Medical Association, 1964, as amended 2004) and International Conference on Harmonisation Good Clinical Practice (GCP) guidelines (International Conference on Harmonisation, 1996). The study was conducted in two sessions between October 2007 and April 2008.

This was a randomized, open-label, controlled study with two study groups, EHCSS-K6 and conventional cigarettes (CC). The study schedule consisted of eight main study visits, screening (Visit 1), two baseline, weekly assessments (Visits 2 and 3), and five post-randomization weekly assessments (Visits 4–8). The whole study duration was approximately 8 weeks (Fig. 1), with the investigational period defined as 5 weeks from the date of randomization (Visit 3/Day 0) to the last study visit (Visit 8/Day 35).

The screening assessment at Visit 1 included the following assessments: physical examination, vital signs, chest X-ray, electrocardiography (ECG), clinical laboratory, drug screening, pregnancy test, and collection of demographic data and smoking history, including the Fagerström Test for Nicotine Dependence (Heatherton et al., 1991). Blood and urine samples were taken for serum chemistry, hematology, serology, and urine analysis.

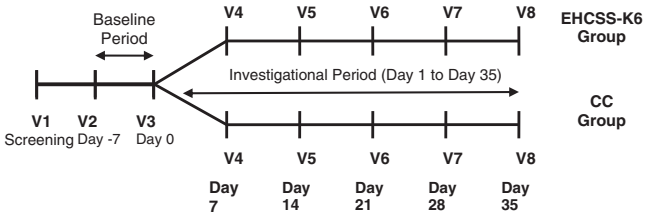

Fig. 1. Study design and assessment schedule. All subjects were scheduled to attend the study site on Visit 1 for screening assessments, Visits 2 and 3 for baseline assessments of biomarkers prior to randomization at Visit 3; and Visits 4 through 8 for study investigations. Study visits were scheduled 7 (±2) days apart.

Randomization of subjects was performed at Visit 3 after completion of the visit-specific assessments using an Interactive Voice Response System (IVRS) (Covance, Waterloo, Belgium). Subjects were randomized to either the EHCSS-K6 group or the CC group, respectively, stratified by gender (40–60% of each gender) and age (50% subjects aged 30–44 years and 50% aged 45–60 years). A randomization ratio of 3:1 (EHCSS-K6:CC) was used to account for possible non-compliance in the EHCSS-K6 group.

Subjects randomized to the CC group continued to smoke their own brand of cigarettes for the duration of the study. Subjects in the EHCSS-K6 group were asked to exclusively smoke the EHCSS-K6 cigarette from Visit 3 until the end of the study. Blinding was not possible due to the differences in physical appearance of the cigarettes to be used.

The assessment schedule of biomarkers over the course of the study consisted of blood sampling (in fasted state) on Visits 2, 3, 5, 7, and 8, and a 24-hour urine collection prior to Visits 3 and 8 (for urinary biomarkers). Carbon monoxide breath testing (Smokerlyzer®, Bedfont Scientific Ltd., Rochester, UK) was performed at all visits from Visit 2 through Visit 8.

Adherence to the study protocol and to Good Clinical Practice was ensured by regular monitoring visits by an independent monitor and by an independent audit of the investigational site.

2.3. Cigarette products and compliance

The EHCSS series-K cigarette was analyzed for tar, nicotine and CO mainstream smoke yields according to International Organization for Standardization (ISO) methods. All study cigarettes were conditioned according to ISO standard 3402 (International Organization for Standardization, 1991). Mainstream smoke from EHCSS cigarettes was generated on a modified smoking machine with a carousel adapted to use the EHCSS series-K lighter. The EHCSS smoke generation conformed with ISO standard 3308 (International Organization for Standardization, 2000a); however, some slight technical deviations were required. Tar, nicotine and CO were determined according to ISO standards 4387, 10315, and 8454, respectively (International Organization for Standardization, 2000b, 2000c; International Organization for Standardization, 1995). The ISO yields as declared on the EHCSS-K6 pack were as follows: 5 mg tar, 0.3 mg nicotine, and 1.0 mg CO.

As EHCSS-K6 cigarettes were not commercially available on the Polish market they were provided free-of-charge to the subjects. Conventional cigarettes were not analyzed or provided to subjects in the CC group, and were purchased by the subjects according to their usual habits.

Subjects in the EHCSS-K6 group were trained on the use of the EHCSS series-K heater at Visit 3 and up to 30 packs (of 20 cigarettes each) of the EHCSS-K6 cigarette were provided at Visits 3–7. Unused cigarettes EHCSS-K6 cigarettes were returned at the following visit.

Subjects were trained on the use of the e-diary (PHT LogPad®, PHT Corporation, Charlestown, MA) and were instructed to document the number of cigarettes smoked each day from Visits 2 through 8, and to record the use of any other tobacco- or nicotine-containing products. These entries were used to assess compliance. In the EHCSS-K6 group, subjects were considered to have good compliance if they smoked $\leqslant$10% CC and no more than 2 CC per day, and did not use any other nicotine- or tobacco-containing products. In the CC group, subjects were considered compliant if they smoked exclusively conventional cigarettes.

2.4. Bioanalytical methods

A detailed sample-handling manual was prepared to describe the preparation, transport, storage, and analysis of samples. All laboratory analyses were performed in a blinded manner without knowledge of the study group assignment.

Biomarkers associated with CVD in smokers are listed in Table 1. Hematology analysis was performed on an automated hematology analyzer (Sysmex XS-800i; Sysmex Europe GmbH, Norderstedt, Germany). *hs*-CRP was determined by particle-enhanced immunological agglutination using anti-CRP antibodies coupled to latex microparticles with a Hitachi 902 chemistry analyser (Roche Diagnostics GmbH, DE-82377 Penzberg, Germany). An automated enzyme-linked immunosorbent assay (ELISA) system (Immunomat™ BASE Plus; Serion Immundiagnostica GmbH, D-97076 Würzburg, Germany) with commercial ELISA kits was used to determine interleukin-6 (IL-6) (Ref. No.: 953 030 192 CE; GenProbe Diaclone SAS, 20250 Besancon, France), oxidized low-density lipoprotein (ox-LDL) (Ref. No.: K7810; Immundiagnostik AG, D-64625 Bensheim, Germany), myeloperoxidase (MPO) (Ref. No.: K6631; Immundiagnostik AG, D-64625 Bensheim, Germany), and von Willibrand factor (vWF) (Ref. No.: 5450201; Technoclone GmbH, 1230 Vienna, Austria). Fibrinogen was determined by the Clauss clotting method on a STA Compact Analyzer (Diagnostica Stago S.A.S., 92602 Asnières sur Seine, France). Urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) and 11-dehydro-thromboxane B_2 (11-$DTXB_2$) were determined by liquid chromatography – tandem mass spectrometry (LC–MS/MS) using an Agilent HP1100 Series HPLC Value System (Agilent Technologies, D-76337 Waldbronn, Germany) equipped with an Applied Biosystems/MDS SCIEX 4000 Qtrap® triple quadrupole mass spectrometer with Applied Biosystems/MDS Sciex Analyst® software version 1.4.2 (Applied Biosystems, Foster City, CA 94404, USA).

Tobacco-specific and tobacco-related biomarkers of exposure (Hecht, 2003; Lindner et al., 2011; Schorp et al., 2012) were determined for the following HPHC (Table 2) using methods validated according to the FDA criteria (Food and Drug Administration, 2001). These included nicotine and five metabolites (expressed as nicotine equivalents: Neq) as a biomarker of exposure for nicotine (Benowitz et al., 1994), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronide conjugates (total NNAL) for NNK (Carmella et al., 2003), monohydroxybutenyl mercapturic acid (MHBMA) for 1,3-butadiene (van Sittert et al., 2000), 3-hydroxypropyl mercapturic acid (3-HPMA) for acrolein (Mascher et al., 2001), *S*-phenyl mercapturic acid (*S*-PMA) for benzene (Medeiros et al., 1997), and 1-hydroxypyrene and its sulfate and glucuronide conjugates (total 1-OHP) for pyrene (Strickland et al., 1996). Urinary concentrations of 2-naphthylamine, 4-aminobiphenyl, and *o*-toluidine were measured directly (Riedel et al., 2006). Some of the instrumentation used (Table 2) differed to that reported in the original methods. Carboxyhemoglobin (COHb) was measured in blood by spectrophotometry (Pojer et al., 1984).

Biomarker analyses were performed at Synevo Clinical Trials, Central Laboratory Services, Gdańsk, Poland (Synevo), Philip Morris Research Laboratories GmbH, Cologne Germany (PMRL), MTZ, and MDS Pharma Services, Fehraltorf, Switzerland (MDS).

2.5. Medications

All previous and ongoing concomitant medications were recorded prior to and during the course of the study. Use of any medication that might have interfered with study results was not allowed; this included anti-inflammatory drugs, hormonal therapies, or nicotine replacement therapy. Paracetamol (acetaminophen) was allowed up to 1500 mg/day.

2.6. Safety evaluations

Safety evaluations were conducted on a weekly basis from Visits 2 to 8 and included adverse events (AEs), concomitant medications, and vital signs. ECG and physical examinations were conducted at Visits 1 and 8. A complete safety laboratory assess-

Table 1
Summary of biomarkers associated with CVD and bioanalytical methods.

Biomarker	Matrix	Method	Lower limit of quantification
White blood cell (WBC) count	Blood	Flow cytometry	0.1×10^9/L
WBC differential	Blood	Flow cytometry	0.1×10^9/L
Platelet count	Blood	DC detection method	10×10^9/L
Red blood cell (RBC) count	Blood	DC detection method	0.02×10^{12}/L
Hemoglobin	Blood	Spectrophotometry	0.1 g/dL
Hematocrit	Blood	Cumulative pulse height detection method	0.1%
High-sensitivity C-reactive protein (*hs*-CRP)	Serum	Immuno-turbidimetry	0.03 mg/L
Interleukin-6 (IL-6)	Serum	ELISA	2 pg/mL
Oxidized low-density lipoprotein (oxLDL cholesterol)	Serum	ELISA	4.13 ng/mL
Myeloperoxidase	Serum	ELISA	1.6 ng/mL
Homocysteine	Serum	Microparticle enzyme immunoassay	0.8 µmol/L
High-density lipoprotein (HDL)	Serum	Photometry	3 mg/dL
Low-density lipoprotein (LDL)	Serum	Photometry	Derived
Total cholesterol	Serum	Photometry	3 mg/dL
von Willebrand factor (vWF)	Plasma	ELISA	0.14 U/mL
Fibrinogen	Plasma	Clot detection	1.5 g/L
Adenosine diphosphate (ADP)-induced platelet aggregation	Plasma	ADP-induced blood platelet aggregation	1%
8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$)	Urine	LC–MS/MS	0.282 nmol/L
11-dehydro-thromboxane B_2 (11-$DTXB_2$)	Urine	LC–MS/MS	0.678 nmol/L

ELISA, enzyme-linked immunosorbent assay; Hct, hematocrit; LC–MS/MS, liquid chromatography–mass spectrometry/mass spectrometry.

Table 2
Summary of smoke constituent biomarkers of exposure and bioanalytical methods.

Smoke constituent	Biomarker	Matrix	Analytical method[a]	Lower limit of quantification
1,3-Butadiene	Monohydroxybutenyl mercapturic acid (MHBMA)	Urine	LC–MS/MS	100 pg/ml
2-Naphthylamine	2-Naphthylamine (2-NA)	Urine	LC–MS/MS	5.0 pg/ml
4-Aminobiphenyl	4-Aminobiphenyl (4-ABP)	Urine	LC–MS/MS	5.0 pg/ml
Acrolein	3-Hydroxypropyl mercapturic acid (3-HPMA)	Urine	LC–MS/MS	35 ng/ml
Benzene	*S*-Phenyl mercapturic acid (*S*-PMA)	Urine	LC–MS/MS	20 pg/ml
Carbon monoxide	Carboxyhemoglobin (COHb)	Blood	Spectrophotometry	0.3% Saturation
Nicotine	Nicotine equivalents (NEq)[c]	Urine	LC–MS/MS	NA[f]
NNK[b]	Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (total NNAL)[d]	Urine	LC–MS/MS	5.0 pg/ml
Pyrene	Total 1-hydroxypyrene (total 1-OHP)[e]	Urine	LC–MS/MS	10 pg/ml
o-Toluidine	*o*-Toluidine (*o*-TOL)	Urine	LC–MS/MS	25 pg/ml

[a] LC–MS/MS, liquid chromatography–tandem mass spectrometry.
[b] NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.
[c] Nicotine equivalents (NEq) were determined as the molar sum of nicotine, cotinine, and *trans*-3′-hydroxycotinine plus their respective glucuronide conjugates.
[d] Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) was determined as the molar sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its *O*-glucuronide conjugate.
[e] Total 1-hydroxypyrene (1-OHP) was determined as the molar sum of 1-hydroxypyrene and its glucuronide and sulfate conjugates.
[f] NA, not applicable.

ment, including hematology, clinical chemistry, and urinalysis was performed at Visits 1 and 8.

2.7. Data analysis

For the purpose of data analysis, the baseline value of biomarkers analyzed in blood was the mean of the values from Visits 2 and 3, and the end-of-study value (EOS) value was the mean of the values from Visits 7 and 8. For all urinary biomarkers, baseline and end-of-study values were as measured at Visits 3 and 8, respectively.

Two analysis sets were defined. The full-analysis set (FAS) population consisted of all subjects who smoked at least one cigarette following randomization, and had valid measurements of the primary biomarkers taken at baseline and at least once post-baseline. The per-protocol (PP) population included subjects who completed the study per protocol with good compliance, with baseline and end-of-study measurements of the primary biomarkers, and without major protocol deviations.

Descriptive statistics were provided for all biomarkers. Results were stratified by study group. Biomarkers were analyzed using the one-sample *t*-test for within-group changes and analysis of covariance (ANCOVA) for between-group differences on reported, untransformed values, if those values were normally distributed. In case of significant deviation from normality of the untransformed data, but not for log-transformed data, both data types were analyzed using the above tests. If non-normality was detected for both data types, statistical analysis was performed using non-parametric methods. The within-study group change from baseline to the end-of-study was analyzed using Wilcoxon rank sum test. The same test was used for the comparison of the two study groups with regard to absolute end-of-study values and changes from baseline to end-of-study.

Analyses for the two primary biomarkers, *hs*-CRP and WBC, were performed on per-protocol and full-analysis-set populations. Analyses for other biomarkers were performed for the full-analysis-set population only.

Since this was an exploratory study, no adjustment for multiplicity was made. All results from inferential statistics should be considered as indicative only. All statistical analyses were performed using SAS® V8.2 (SAS Institute Inc., Cary, NC, USA) according to a predefined statistical analysis plan.

2.8. Determination of sample size

Sample-size estimation was based on the observed variability in a previous study (Roethig et al., 2008) for the two primary biomarkers, *hs*-CRP and WBC. The estimated sample size for this study was 80 subjects per study group to allow detection of effect size of $\Delta = 0.47$ for *hs*-CRP with a power of 80% at a significance level $\alpha = 0.05$ using a two-sided, two-sample *t*-test. The anticipated effect size for WBC count ($\Delta = 0.66$) was greater than that of *hs*-CRP, so the calculated sample size for *hs*-CRP was considered to be large enough for WBC count. The sample size was increased in the EHCSS-K6 study group from 80 to 240 subjects to account for possible non-compliance.

2.9. Adverse events, medical history, and concomitant medication

Adverse events (AEs) and medical history were coded using the Medical Dictionary for Regulatory Activities (MedDRA Version 7.0). Medications were coded according to the WHO Drug Reference List (WHO Drug Dictionary, Uppsala Monitoring Center, 2003 quarter 4).

3. Results

3.1. Demographics and other baseline characteristics

A total of 338 subjects were enrolled in the study and 316 were randomized (Fig. 2). Of the 316 randomized subjects, 237 were randomized to the EHCSS-K6 and 79 to the CC group study group. For the 22 subjects not randomized, the most common reasons were AEs prior to randomization or violation of selection criteria. The study was completed by 309 subjects, with 234 and 75 subjects for the EHCSS-K6 and CC groups, respectively. There were 7 subjects who did not complete the study. In the EHCSS-K6 group, 2 subjects were withdrawn as they did not attend study visits and 1 subject withdrew consent. In the CC group, 2 subjects were withdrawn as they did not attend study visits, 1 subject was withdrawn due to influenza and 1 subject for violation of selection criteria.

Of the 316 subjects, 161 (51%) were male and 155 (49%) were female. The mean age of all subjects was 44 years old, with 52% of subjects in the 30- to 44-year age group and 48% in the 45- to 60-year age group. The body mass index (BMI) classification showed 47.2% of subjects to have normal weight, 36.7% to be overweight, 13.6% to be obese, and 2.5% to be underweight (Table 3).

The Fagerström Test for Nicotine Dependence (FTND) scores were similar for subjects subsequently randomized to both study groups, with a mean of 5.9 ± 2.0 in both study groups. Smoking history was similar for both groups. The majority of subjects reported a smoking history of greater than 20 years, 61% and 68% for the EHCSS-K6 and CC groups, respectively.

Three major protocol deviations were observed in the study, all in the EHCSS-K6 group. Two subjects did not fast prior to blood

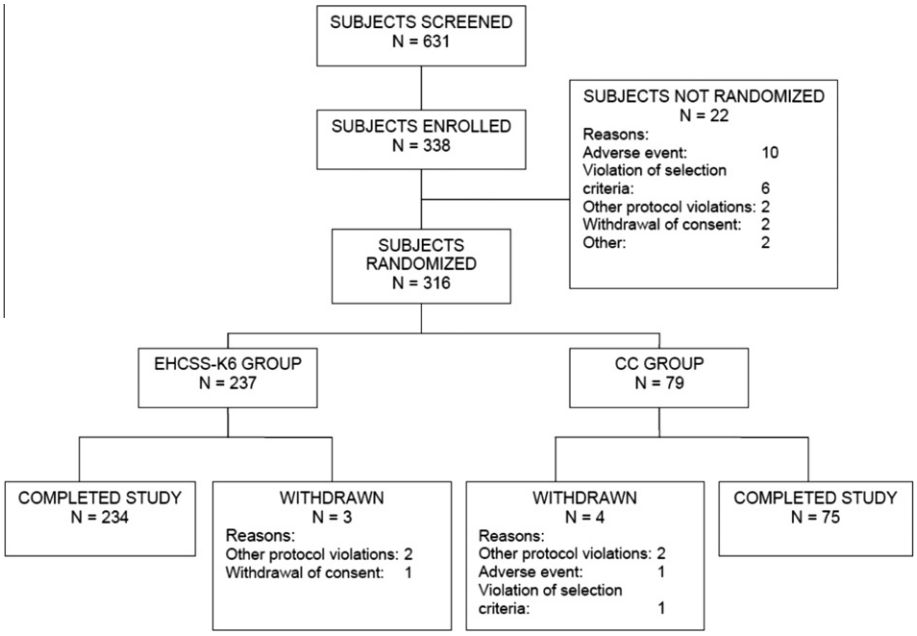

Fig. 2. Subject disposition. A total of 338 subjects were enrolled on the study, of which 316 were randomized, and the study was completed by 309 subjects (234 and 75 subjects in the EHCSS-K6 and CC groups, respectively). EHCSS-K6, EHCSS series-K heater and cigarette (5 mg tar, 0.3 mg nicotine, and 1.0 mg CO); CC, conventional cigarette.

Table 3
Subject demographics by study group as measured at baseline.

Variable and statistics	Study group[a]		
	EHCSS-K6	CC	Overall
Number of subjects (*N*)	237	79	316
Age (years, mean ± SD)	43.5 ± 8.0	43.9 ± 8.2	43.6 ± 8.1
Gender (N, % of total)			
Male	121 (51)	40 (51)	161 (51)
Female	116 (49)	39 (49)	155 (49)
BMI (kg/m^2 mean ± SD)[b]	25.15 ± 4.58	23.93 ± 3.29	24.84 ± 4.32
Classification			
Underweight (N, %)	6 (2.5)	2 (2.5)	8 (2.5)
Normal weight (N, %)	106 (44.7)	43 (54.4)	149 (47.2)
Overweight (N, %)	87 (36.7)	29 (36.7)	116 (36.7)
Obese (N, %)	38 (16.0)	5 (6.3)	43 (13.6)

[a] Groups abbreviated as: EHCSS-K6, EHCSS series-K heater and cigarette (5 mg tar, 0.3 mg nicotine, and 1.0 mg CO); CC, conventional cigarette.
[b] BMI, body mass index.

Table 4
Daily cigarette consumption by group and study week.

Study week	Study group[a]			
	EHCSS-K6		CC	
	N	Mean ± SD	*N*	Mean ± SD
−1[b]	237	25 ± 6	79	25 ± 6
1	236	33 ± 5	77	24 ± 1
2	236	37 ± 1	75	24 ± 1
3	236	36 ± 5	75	23 ± 2
4	235	36 ± 4	75	23 ± 1
5	235	40 ± 2	75	23 ± 1
All[c]	236	38 ± 3	77	23 ± 1

[a] Groups abbreviated as: EHCSS-K6, EHCSS series-K heater and cigarette (5 mg tar, 0.3 mg nicotine, and 1.0 mg CO); CC, conventional cigarette.
[b] All subjects smoked CC during Week -1.
[c] Average daily cigarette consumption on Weeks 1–5.

sampling and 1 subject who used nonsteroidal anti-inflammatory drugs within 2 weeks prior to screening. Minor deviations, related to study timing or procedure, occurred in both study groups with comparable frequencies and types of deviations reported.

3.2. Cigarette consumption and compliance

The mean number of cigarettes smoked daily for both groups were similar at baseline: 25 cigarettes per day (CPD) (Table 4). In the EHCSS-K6 group, the average daily consumption increased over the course of the study from 33 CPD in Week 1 to 40 CPD in Week 5, with an overall mean of 38 CPD across Visits 3–8. This increasing trend for the EHCSS-K6 study group was consistent throughout the subgroups for age and gender. For the CC group, the overall mean across Visits 3–8 was 23 CPD.

For the full-analysis-set population, the mean compliance for the EHCSS-K6 group, based on self-reporting in the e-diary, ranged from 99% to 100%. A total of 13 subjects in this group reported smoking CC on at least one occasion. Compliance rates for subjects in the CC study group who continued to smoke their own type of CC, based on self-reported data, ranged from 97.3% to 98.7%. Three subjects in the CC group reported the use of other tobacco- or nicotine-containing products.

3.3. Primary biomarkers

Baseline values were similar for the primary biomarkers *hs*-CRP and WBC count. For the primary analysis of between-group comparisons at the end-of-study, no statistically significant differences were observed.

Within-group comparisons showed reductions in median serum *hs*-CRP from baseline values (1.37 mg/L) to the end of study (1.11 mg/L) for the EHCSS-K6 study group and from 1.18 to 0.85 mg/L in the CC group. However, the results using mean values were different (Table 5), and it is therefore unclear whether there was any real change.

There were no statistically significant differences for WBC counts between study groups at the end-of-study. A reduction in mean WBC counts from baseline ($7.09 \pm 1.73 \times 10^9$/L) to the end-of-study ($6.90 \pm 1.64 \times 10^9$/L) occurred in the EHCSS-K6 group.

Table 5
Summary of overall high sensitivity C-reactive protein (*hs*-CRP) concentrations at baseline and end of study, and change from baseline to end of study by study group (PP population).

Time point	*hs*-CRP mg/L	Study group[a]	
		EHCSS-K6	CC
Baseline[b]	Mean ± SD	2.20 ± 2.71	2.02 ± 2.49
	Median (min., max.)	1.4 (0.2, 23.2)	1.2 (0.3, 11.7)
End of study[c]	Mean ± SD	2.04 ± 2.80	2.27 ± 5.34
	Median (min., max.)	1.1 (0.2, 27.5)	0.8 (0.2, 35.5)
Change from baseline	Mean ± SD	−0.15 ± 3.41	0.25 ± 5.15
	Median (min., max.)	−0.1 (−21.4, 26.0)[d]	−0.1 (−11.2, 30.1)

[a] Groups abbreviated as: EHCSS-K6, EHCSS series-K heater and cigarette (5 mg tar, 0.3 mg nicotine, and 1.0 mg CO); CC, conventional cigarette.
[b] Baseline values are a mean of Visits 2 and 3.
[c] End-of-study values are a mean of Visits 7 and 8.
[d] Statistically different from baseline ($p \leqslant 0.01$).

Table 6
Summary of overall white blood cell (WBC) counts at baseline and end of study, and change from baseline to end of study by study group (PP population).

Time point	WBC count ($\times 10^9$/L)	Study group[a]	
		EHCSS-K6	CC
Baseline[b]	Mean ± SD	7.09 ± 1.73	7.00 ± 1.63
	Median (min., max.)	6.9 (3.4, 12.7)	6.8 (4.2, 10.9)
End of study[c]	Mean ± SD	6.90 ± 1.64[d]	6.94 ± 1.60
	Median (min., max.)	6.6 (3.7, 14.0)	6.9 (4.4, 11.0)
Change from baseline	Mean ± SD	−0.20 ± 1.23	−0.06 ± 0.94
	Median (min., max.)	−0.2 (−5.5, 5.3)	−0.1 (−2.7, 3.7)

[a] Groups abbreviated as: EHCSS-K6, EHCSS series-K heater and cigarette (5 mg tar, 0.3 mg nicotine, and 1.0 mg CO); CC, conventional cigarette.
[b] Baseline values are a mean of Visits 2 and 3.
[c] End-of-study values are a mean of Visits 7 and 8.
[d] Statistically different from baseline ($p \leqslant 0.01$).

This tendency was observed to a smaller extent in the CC group with mean WBC baseline levels of $7.00 \pm 1.63 \times 10^9$/L, and end-of-study levels of $6.94 \pm 1.60 \times 10^9$/L (Table 6).

3.4. Other biomarkers associated with cardiovascular disease

Other biomarkers associated with CVD were found to be comparable at baseline. A summary of findings for these biomarkers is presented in (Table 7).

A statistically significant reduction ($p < 0.05$) in neutrophil counts from $4.11 \pm 1.35 \times 10^9$/L to $3.92 \pm 1.28 \times 10^9$/L from baseline to the end-of-study within the EHCSS-K6 group was observed. An increase in high-density lipoprotein (HDL) cholesterol from 59.0 ± 16.3 mg/dL to 63.9 ± 17.3 mg/dL from baseline to the end-of-study in the EHCSS-K6 group was found, but not in the CC group. HDL was also higher in the EHCSS-K6 group (63.9 ± 17.3 mg/dL) compared to the CC group at the end-of-study (62.3 ± 16.1 mg/dL). Within-group analysis for baseline to end-of-study values revealed statistically significant decreases in the EHCSS-K6 group of red blood cells (RBC, by 0.08×10^{12}/L; $p \leqslant 0.001$), hemoglobin (by 0.29 g/dL; $p \leqslant 0.001$), and hematocrit (by 0.92%; $p \leqslant 0.001$). End-of-study values of all three parameters in the EHCSS-K6 groups were lower than in the CC group. Statistical comparisons between study arms (EHCSS-K6 *vs*. CC) at the end of the study showed statistically significant decreases in the EHCSS-K6 compared to the CC study arms for RBC ($p \leqslant 0.01$), hematocrit ($p \leqslant 0.001$), and hemoglobin ($p \leqslant 0.001$).

A median decrease of 6 ng/mL was observed in myeloperoxidase levels in the EHCSS-K6 group at the end-of-study, compared to an increase of 23 ng/mL in the CC group. Neither of these changes was statistically significant. Urinary 11-DTXB$_2$ excretion decreased from baseline to the end-of-study in the EHCSS-K6 group from 14.47 ± 8.49 pg/24 h to 13.25 ± 8.68 pg/24 h. Although there was a similar tendency in the CC group, this was of a smaller magnitude. All other changes in biomarkers in this study were either non-specific to the EHCSS-K6 group or were not considered to be clinically relevant.

3.5. Biomarkers of exposure

Study groups were comparable with regards to levels of biomarkers of exposure at baseline. All biomarkers of exposure measured in this study were significantly reduced from baseline to end-of-study in the EHCSS-K6 study group. Furthermore, at the end-of-study all biomarkers of exposure were substantially lower in the EHCSS-K6 group than in the CC group. A summary of the biomarker of exposure data is presented in Table 8.

In the EHCSS-K6 group a reduction in the percent saturation of COHb from 4.35% ± 1.59% to 2.32% ± 1.07%, a reduction in total NNAL from 346.1 ± 226.6 to 186.3 ± 147.1 ng/24 h, and a reduction in *S*-PMA from 5.61 ± 7.64 to 2.00 ± 2.53 μg/24 h were observed. Total nicotine equivalent (Neq) levels decreased from 17.93 ± 8.30 to 14.66 ± 7.90 mg/24 h from baseline to end-of-study in the EHCSS-K6 group. A smaller decrease by 1.75 mg/24 h of Neq levels was observed in the CC group, while changes in all other biomarkers of exposure were not significant.

3.6. Safety evaluations/serious adverse events

Similar percentages of subjects reported AEs: 53% and 58% in the EHCSS-K6 and CC groups, respectively. The most commonly reported AEs that were considered related to the EHCSS-K6 cigarette were dry mouth, dry throat, cough and diarrhea, which occurred in 0.4–1.3% of subjects.

Three serious adverse events (SAEs) were reported. A deep vein thrombosis in the EHCSS-K6 group which occurred approximately 1 week after the end of the study when the subject had restarted smoking CC. This event was considered by the investigator to be possibly related to the EHCSS-K6 cigarette, as cigarette smoking is a known risk factor for this condition. A post-traumatic splenic injury (EHCSS-K6 study group) that occurred after the end of the study, and was not considered to be related to the study product. A myocardial infarction that occurred prior to randomization. There were no apparent differences between groups in any of the clinical laboratory values, vital signs, ECG parameters, or physical examinations during the study.

4. Discussion

This study was designed to compare biomarkers associated with CVD and biomarkers of exposure to selected cigarette smoke HPHC in subjects switching to the EHCSS-K6 cigarette compared to those continuing to smoke conventional cigarettes in real-life, actual use conditions. In a similar randomized clinical trial conducted over 12 months with an earlier prototype of the EHCSS and group of smokers who used a 6 mg tar conventional cigarette (Roethig et al., 2008), mean cigarette consumption increased from 24.3 and 23.3 CPD at baseline to 45.1 and 31.0 after Week 4, respectively. Very similar to that reported in the current study in which smokers of the EHCSS-K increased mean consumption from 25 CPD at baseline while smoking own-brand cigarettes to 38 CPD when smoking EHCSS-K6 (Table 4), while no significant change in cigarette consumption occurred in smokers who continued to smoker their own brand of CC (25 CPD). Providing EHCSS cigarettes

Table 7
Summary of biomarkers associated with cardiovascular disease.

	Study group[a]					
	EHCSS-K6			CC		
Biomarkers associated with cardiovascular disease	Baseline[b] (*N* = 236)	EOS[c] (*N* = 235)	Change[d]	Baseline[b] (*N* = 77)	EOS[c] (*N* = 75)	Change[d]
Neutrophils ($\times 10^9$/L)						
Mean ± SD	4.11 ± 1.35	3.92 ± 1.28	−0.187 ± 1.105	3.92 ± 1.37	3.91 ± 1.35	−0.056 ± 0.772
Median (min., max.)	3.9 (1.5, 9.1)	3.7 (1.6, 10.7)	−0.2 (−5.3, 5.6)	3.7 (1.6, 7.8)	3.8 (1.5, 7.7)	−0.2 (−1.4, 2.6)
Lymphocytes ($\times 10^9$/L)						
Mean ± SD	2.13 ± 0.57	2.13 ± 0.55	−0.003 ± 0.331	2.10 ± 0.52	2.12 ± 0.43	0.004 ± 0.308
Median (min., max.)	2.1 (1.0, 4.3)	2.0 (1.0, 3.8)	0.0 (−1.1, 1.2)	2.0 (1.0, 4.5)	2.1 (1.2, 3.9)	(0.0, −0.8, 0.6)
Monocytes ($\times 10^9$/L)						
Mean ± SD	0.646 ± 0.179	0.646 ± 0.177	−0.000 ± 0.107	0.653 ± 0.160	0.661 ± 0.175	0.005 ± 0.114
Median (min., max.)	0.63 (0.28, 1.46)	0.63 (0.31, 1.36)	−0.01 (−0.30, 0.44)	0.62 (0.39, 1.19)	0.65 (−0.35, 1.27)	−0.01 (−0.32, 0.36)
Eosinophils ($\times 10^9$/L)						
Mean ± SD	0.181 ± 0.099	0.184 ± 0.104	0.004 ± 0.067	0.212 ± 0.118	0.225 ± 0.121	0.010 ± 0.057
Median (min., max.)	0.17 (0.0, 0.54)	0.17 (0.0, 0.63)	0.00 (−0.26, 0.26)	0.19 (0.04, 0.84)	0.20 (0.04, 0.84)	0.01 (−0.15, 0.19)
Basophils ($\times 10^9$/L)						
Mean ± SD	0.026 ± 0.012	0.027 ± 0.014	0.001 ± 0.009	0.026 ± 0.011	0.028 ± 0.013	0.002 ± 0.008
Median (min., max.)	0.03 (0.01, 0.07)	0.03 (0.01, 0.08)	0.00 (−0.03, 0.06)[d]	0.03 (0.01, 0.06)	0.03 (0.01, 0.07)	0.00 (−0.02, 0.02)
HDL (mg/dL)						
Mean ± SD	59.0 ± 16.3	63.9 ± 17.3	5.0 ± 8.5[f]	61.5 ± 16.3	62.3 ± 16.1	0.9 ± 7.1
Median (min., max.)	57 (27, 136)	63 (28, 123)	5 (−54, 39)	54 (34, 116)	60 (35, 113)	1 (−26, 20)
LDL (mg/dL)						
Mean ± SD	121.5 ± 32.9	118.6 ± 31.2	−3.4 ± 18.6[g]	120.8 ± 28.8	118.3 ± 30.1	−3.0 ± 14.0
Median (min., max.)	122 (38, 212)	116 (33, 219)	−4 (−59, 49)	119 (56, 185)	119 (57, 194)	−5 (−41, 32)
Total cholesterol (mg/dL)						
Mean ± SD	204.5 ± 35.3	207.0 ± 33.2	2.3 ± 21.1	203.6 ± 32.4	203.1 ± 31.2	−1.2 ± 17.0
Median (min., max.)	203 (113, 333)	206 (116, 301)	3 (−88, 66)	201 (144, 274)	198 (57, 194)	−1 (−46, 35)
RBC count ($\times 10^{12}$/L)						
Mean ± SD	4.56 ± 0.41	4.48 ± 0.41	−0.083 ± 0.175[e]	4.55 ± 0.39	4.54 ± 0.39	−0.016 ± 0.143
Median (min., max.)	4.5 (3.7, 5.7)	4.4 (3.6, 5.5)	−0.1 (−1.2, 0.3)	4.5 (3.8, 5.4)	4.6 (3.6, 5.5)	(−0.4, 0.3)
Hemoglobin (g/dL)						
Mean ± SD	14.29 ± 1.16	14.00 ± 1.18	−0.29 ± 0.51[e]	14.28 ± 1.28	14.21 ± 1.32	−0.06 ± 0.42
Median (min., max.)	14.3 (−10.7, 17.3)	14.0 (10.3, 16.7)	−0.3 (−3.3, 0.8)	14.4 (10.5, 16.5)	14.3 (10.0, 16.5)	0.0 (−1.1, 0.9)
Hematocrit (%)						
Mean ± SD	42.67 ± 3.16	41.75 ± 3.08	−0.92 ± 1.46[e]	42.58 ± 3.41	42.44 ± 3.50	−0.10 ± 1.26
Median (min., max.)	42.6 (35.6, 50.4)	41.4 (34.6, 48.7)	−0.8 (−9.0, 2.2)	42.9 (34.0, 48.7)	43.1 (33.0, 48.6)	0.0 (−3.5, 2.3)
IL−6 (pg/mL)						
Mean ± SD	1.39 ± 0.83	1.65 ± 3.00	0.25 ± 2.96	1.45 ± 1.04	1.41 ± 1.04	−0.03 ± 1.26
Median (min., max.)	1.0 (1.0, 5.2)	1.0 (1.0, 39.5)	0.0 (−3.9, 37.3)	1.0 (1.0, 6.4)	1.0 (1.0. 7.2)	0.0 (−4.4, 6.2)
Ox LDL (ng/mL)						
Mean ± SD	179.0 ± 485.7	184.2 ± 454.3	4.81 ± 188.5	167.3 ± 396.0	177.4 ± 365.9	6.21 ± 328.2
Median (min., max.)	36.7 (2.1, 3410)	59.0 (2.1, 4535)	15.5 (−1472, 1280)[f]	47.2 (2.1, 2930)	58.6 (2.1, 2260)	8.8 (−2158, 1028)[g]
Myeloperoxidase (ng/dL)						
Mean ± SD	252.4 ± 107.6	245.5 ± 114.1	−6.5 ± 86.0	239.1 ± 102.6	255.7 ± 113.4	17.1 ± 87.4
Median (min., max.)	223.5 (8.1, 676)	219.0 (3.0, 807)	−6.3 (−271.5, 278)	224.5 (90.8, 635)	231.0 (67.6, 575)	23.0 (−220, 325)
8-epi-$PGF_{2\alpha}$ (pg/24 h)						
Mean ± SD	8.98 ± 4.14	9.08 ± 4.88	0.06 ± 4.57	8.63 ± 3.27	8.40 ± 4.30	−0.32 ± 3.73
Median (min., max.)	8.4 (1.1, 26.2)	7.8 (1.1, 29.8)	−0.17 (−19.5, 21.1)	8.2 (1.4, 18.4)	7.6 (1.9, 22.1)	−0.24 (−8.3, 12.1)
Platelet count ($\times 10^9$/L)						
Mean ± SD	227.6 ± 51.4	230.5 ± 50.7	2.6 ± 23.3	221.7 ± 46.6	225.2 ± 51.0	2.8 ± 20.9
Median (min., max.)	222 (122, 393)	224 (118, 417)	1 (−74, 108)	217 (133, 347)	223 (118, 338)	−1 (−43, 58)
Homocysteine (mol/L)						
Mean ± SD	11.60 ± 3.22	11.76 ± 3.48	0.15 ± 1.56	12.21 ± 3.77	12.44 ± 3.86	0.22 ± 2.20
Median (min., max.)	10.9 (7.0, 30.8)	11.2 (6.8, 34.7)	0.2 (−3.8, 10.7)	11.3 (7.1, 31.5)	11.7 (8.0, 30.4)	0.3 (−6.3, 12.3)
vWF (U/l)						
Mean ± SD	0.96 ± 0.31	0.81 ± 0.22	−0.150 ± 0.217[f]	0.95 ± 0.29	0.77 ± 0.22	−0.171 ± 0.207
Median (min., max.)	0.9 (0.4, 2.5)	0.8 (0.3, 1.7)	−0.1 (−1.7, 0.5)	0.9 (0.3, 1.8)	0.8 (0.3, 1.4)	−0.1 (−1.7, 0.5)
Fibrinogen (g/L)						
Mean ± SD	3.6 ±(0.63	3.56 ± 0.62	−0.040 ± 0.465	3.54 ± 0.72	3.60 ± 0.91	0.060 ± 0.537
Median (min., max.)	3.3 (2.2, 5.3)	3.5 (2.3, 5.8)	−0.1 (−1.3, 2.6)	3.5 (2.2, 6.0)	3.5 (2.1, 7.0)	0.0 (−1.3, 2.7)
11-$DTXB_2$ (pg/24 h)						
Mean ± SD	14.47 ± 8.49	13.25 ± 8.68	−1.18 ± 6.97	13.55 ± 5.62	13.33 ± 6.78	−0.25 ± 5.11
Median (min., max.)	12.7 (1.7, 81.0)	11.4 (1.2, 81.0)	−1.3 (−23.8, 34.1)[f]	13.6 (1.7, 28.1)	12.4 (1.6, 42.6)	−0.4 (−13.2, 20.9)

Table 7 (*continued*)

Biomarkers associated with cardiovascular disease	Study group[a]					
	EHCSS-K6			CC		
	Baseline[b] (*N* = 236)	EOS[c] (*N* = 235)	Change[d]	Baseline[b] (*N* = 77)	EOS[c] (*N* = 75)	Change[d]
ADP-induced platelet aggregation: slope						
Mean ± SD	117.2 ± 19.9	120.2 ± 21.2	2.9 ± 19.5[d]	117.4 ± 22.5	120.5 ± 23.1	2.9 ± 16.2
Median (min., max.)	116 (68, 192)	117 (50, 189)	3 (−142, 68)	115 (85, 187)	118 (85, 193)	3 (−39, 45)
ADP-induced platelet aggregation: amplitude (%)						
Mean ± SD	76.6 ± 5.0	78.4 ± 5.3	1.7 ± 5.5	77.2 ± 5.2	78.8 ± 4.3	1.6 ± 4.6
Median (min., max.)	78 (59, 86)	79 (39, 100)	2 (−28, 25)[f]	78 (61,86)	79 (66, 86)	1 (−10, 13)[g]

Abbreviations: ADP, adenosine diphosphate; CC, conventional cigarettes; 11-DTXB$_2$, 11-dehydro-thromboxane B$_2$; EHCSS-K6, electrically heated cigarette smoking system K6; EOS, end of study; 8-epi-PGF$_{2\alpha}$, 8-epi-prostaglandin F$_{2\alpha}$; HDL, high-density lipoprotein; IL-6, interleukin-6; LDL, low-density lipoprotein; RBC, red blood cell count; oxLDL, oxidized low-density lipoprotein; vWF, von Willebrand factor.

[a] Groups abbreviated as: EHCSS-K6, EHCSS series-K heater and cigarette (5 mg tar, 0.3 mg nicotine, and 1.0 mg CO); CC, conventional cigarette.

[b] Baseline measures are mean values of Visits 2 and 3 and all subjects smoked conventional cigarettes.

[c] End-of-study values are mean values of Visits 7 and 8.

[d] Change from baseline to end of study.

[e] Statistically different from baseline ($p \leqslant 0.05$).

[f] Statistically different from baseline ($p \leqslant 0.001$).

[g] Statistically different from baseline ($p \leqslant 0.01$).

free-of-charge cannot solely explain the increase in cigarette consumption in the EHCSS group which could also be due to the reduced number of eight puffs the subjects could obtain using the EHCSS (Werley et al., 2008; Schorp et al., 2012), while users of CC average 12–13 puffs per cigarette (Perkins et al., 2012).

In the present study, no major changes were seen in the primary biomarkers, *hs*-CRP, or WBC count. However, there were reductions, albeit not significant, within the EHCSS-K6 group from baseline to the end-of-study for both parameters.

The choice of both primary biomarkers was based on a previous clinical study in which a non-significant reduction in serum *hs*-CRP and a significant reduction in WBC count were observed over a 12-month study period in subjects who switched from smoking CC to an EHCSS (Roethig et al., 2008). The serum concentrations of *hs*-CRP were noted to be highly variable, and therefore a larger sample size than planned would have been needed in order to detect differences in this biomarker. Post-hoc sample size calculations indicated that, with the variability reported in this study, at least 312 subjects in each group would be necessary to detect the difference in *hs*-CRP that was anticipated in the current study. There is evidence to suggest that the observed variability may be a reflection of the sensitivity of *hs*-CRP to even mild levels of inflammation (Zedler et al., 2006). A study of smoking cessation evaluated markers of inflammation indicated that inflammatory markers such as *hs*-CRP resolve slowly over time even with complete cessation (Bakhru and Erlinger, 2005). Given the long resolution time, contradictory information regarding the effects of smoking on *hs*-CRP and acute elevations not specific to current smoking behaviors, *hs*-CRP would not be recommended as a primary endpoint in studies assessing tobacco products over short study periods.

WBC count has also been implicated as a marker for early atherosclerotic disease, and an independent marker of elevated risk for CVD events (Calapai et al., 2009; Kannel et al., 1992; Lavi et al., 2007). In highly controlled clinical studies in which smokers switched from smoking CC to EHCSS, a significant reduction in WBC count was reported within 3 days suggesting a rapid reduction in inflammation (Roethig et al., 2010). However, under less controlled conditions, statistically significant reductions in WBC count have been reported to occur much later: 4 weeks on smoking cessation (Abel et al., 2005) and 7 weeks on smoking cessation (Hatsukami et al., 2005). Although WBC count is clinically relevant and likely to be a suitable biomarker for measuring inflammation in studies of tobacco products, the current study duration may have been too short to evaluate changes in WBC count in subjects switching to smoke EHCSS-K6 under non-controlled, actual use ambulatory conditions.

Low HDL levels have been associated with smoking and also with an increased risk of CVD (Benowitz, 2003). The reversibility of HDL with smoking cessation is well documented, and recent evidence now suggests that this biomarker may also be reversible with reduction of smoking (Andrews and Tingen, 2006; Benowitz, 2003; Hatsukami et al., 2005). An increase in HDL levels has been observed as early as 1 week following smoking cessation, and after only 2 weeks in studies investigating reduction in cigarette consumption (Benowitz, 2003; Eliasson et al., 2001; Hatsukami et al., 2005; Minami et al., 2002). In a previous assessment of an EHCSS, these increases were maximal at 3 months and were sustained over the course of a 12-month study period (Roethig et al., 2008). The HDL increase observed in this study was similar to that reported in previous studies in both time and magnitude (Hatsukami et al., 2005). The rise of HDL levels of 5 mg/dL (approximately 8%) for the EHCSS-K6 group in the present study is within the reported 5–15% range typically reported with the use of statins for reducing CVD risk (Kapur et al., 2008). This increase may represent a beneficial shift, since there is evidence to support a 2% decrease in CVD risk for every 1 mg/dL increase in HDL levels (Kapur et al., 2008). These results support previous findings that switching from CC to smoke an EHCSS may alter lipid profiles in a favorable manner even when smokers increased the number of cigarettes smoked (Roethig et al., 2008).

Higher levels of hematocrit and hemoglobin have been demonstrated in smokers, and these increases are likely to be compensatory for exposure to CO (Roethig et al., 2008). Increased binding of CO to hemoglobin has been linked to the increased red cell mass, hematocrit, and hemoglobin concentrations observed in smokers that may contribute to a hypercoagulable state (Benowitz, 2003). The present study supports previous EHCSS investigations in which these parameters were reduced in combination with a significant reduction in CO exposure as measured by COHb (Roethig et al., 2007, 2008). Previous investigations with EHCSS products have found as much as 90% reductions in COHb levels, in as few as 8 days using the EHCSS cigarette (Roethig et al., 2005, 2007).

Table 8
Summary of biomarkers of exposure at baseline and end of study (FAS population).

Biomarker of exposure	Study group[a]					
	EHCSS-K6			CC		
	Baseline[b] (*N* = 236)	EOS[c] (*N* = 235)	Change[d]	Baseline[b] (*N* = 77)	EOS[c] (*N* = 75)	Change[d]
COHb (%)						
Mean ± SD	4.35 ± 1.59	2.32 ± 1.07	−2.02 ± 1.41	4.70 ± 1.67	4.90 ± 1.57	0.22 ± 0.91
Median (min., max.)	4.2 (1.1, 9.8)	1.9 (1.1, 7.7)	−2.0 (−8.1, 1.7)[e]	4.7 (1.4, 8.2)	5.1 (1.3, 8.5)	0.1 (−1.9, 3.4)
Neq/24 h (mg/24 h)						
Mean ± SD	17.93 ± 8.30	14.66 ± 7.90	−3.21 ± 6.55	19.75 ± 8.25	17.84 ± 7.98	−1.75 ± 5.42
Median (min., max.)	17.3 (0.4, 49.3)	13.3 (0.4, 45.7)	−3.3 (−26.3, 22.9)[e]	19.0 (2.9, 41.7)	18.1 (3.1, 45.9)	−0.9 (−15.6, 14.5)[f]
3-HPMA (mg/24 h)						
Mean ± SD	2.45 ± 1.66	1.93 ± 1.18	−0.51 ± 1.59	2.31 ± 1.12	2.52 ± 1.41	0.22 ± 1.06
Median (min., max.)	2.2 (0.1, 14.0)	1.7 (0.1, 7.1)	−0.3 (−11.5,4.0)[e]	2.2 (0.4, 5.5)	2.2 (0.4, 8.1)	0.0 (−3.7, 4.8)
S-PMA (μg/24 h)						
Mean ± SD	5.61 ± 7.64	2.00 ± 2.53	−3.60 ± 6.69	5.66 ± 3.67	5.38 ± 3.61	−0.30 ± 2.57
Median (min., max.)	4.4 (0.2, 106.9)	1.2 (0.2, 23.4)	−2.5 (−89.8, 9.1)[f]	5.0 (0.54, 18.3)	5.0 (0.3, 19.3)	0.0 (−10.2, 4.5)
MHBMA (μg/24 h)						
Mean ± SD	8.53 ± 6.48	4.01 ± 4.05	−4.51 ± 5.08	9.52 ± 6.34	8.18 ± 5.26	−1.38 ± 3.96
Median (min., max.)	7.6 (0.1, 35.5)	2.7 (0.2, 25.8)	−3.7 (−29.0, 7.4)[e]	8.9 (0.4, 30.1)	7.7 (0.5, 25.3)	−0.7 (−14.4, 7.8)[g]
NNAL (ng/24 h)						
Mean ± SD	346.1 ± 226.6	186.3 ± 147.1	−160.3 ± 179.7	369.3 ± 213.3	358.3 ± 222.4	−14.4 ± 134.9
Median (min., max.)	316.8 (9.5, 1653.3)	325.5 (49.1, 3803)	−132 (−1456, 301.5)[e]	378.6 (15.0, 1055.2)	318.6 (53.9, 1021.4)	−11.6 (−359.2, 338.8)
1-OHP (ng/24 h)						
Mean ± SD	614.2 ± 766.0	450.2 ± 469.8	−164.3 ± 747.5	514.6 ± 334.8	547.9 ± 364.6	29.5 ± 324.3
Median (min., max.)	492.8 (137.3, 10929)	148.8 (8.8, 939.3)	−161 (−8740, 3525)[e]	460.5 (123.9, 2216)	488.1 (104.5, 2085)	14 (−1698, 1523)
4-ABP (ng/24 h)						
Mean ± SD	27.12 ± 16.83	13.28 ± 19.23	−13.80 ± 20.29	29.24 ± 15.81	28.71 ± 15.40	−0.57 ± 11.15
Median (min., max.)	23.8 (1.3, 84.6)	8.8 (1.2, 243.0)	−13.6 (−77.4, 179.3)[e]	29.8 (3.2, 82.6)	25.8 (3.9, 71.7)	−1.7 (−18.9, 38.5)
o-Toluidine (ng/24 h)						
Mean ± SD	302.0 ± (159.8)	184.0 ± (124.1)	−116.7 ± (146.6)	313.0 ± (167)	334.4 ± (464.1)	−22.9 ± (468.0)
Median (min., max.)	275.8 (34.5, 1222.7)	145.0 (34.9, 803.5)	−114 (−846.5, 445.1)[e]	294.8 (77.1, 1018.9)	255.1 (60.6, 4104.0)	−20.5 (−644.5, 3888.9)[g]
2-NA (ng/24 h)						
Mean ± SD	35.23 ± 17.35	17.05 ± 14.30	−18.28 ± 17.08	38.14 ± 37.65	37.65 ± 17.97	−0.26 ± 11.7
Median (min., max.)	35.2 (2.5, 91.2)	12.1 (2.2, 81.5)	−18.5 (−74.5, 57.8)[e]	37.7 (1.3, 87.0)	36.7 (5.5, 80.6)	−1.3 (−24.2, 31.6)
Breath CO (ppm)[h]						
Mean ± SD	11.4 ± 6.3	4.6 ± 4.3	−6.7 ± 6.1	12.4 ± 6.7	13.6 ± 7.0	1.3 ± 6.2
Median (min., max.)	11 (0, 34)	4 (0, 20)	−6 (−31, 7)	11 (0, 30)	14 (0, 30)	0 (−13, 21)

Abbreviations: 4-ABP, 4-aminobiphenyl; CO, carbon monoxide; COHb, carboxyhemoglobin; EOS, end of study; FAS, full-analysis set population; 3-HPMA, 3-hydroxypropyl mercapturic acid; MHBMA, monohydroxybutenyl mercapturic acid; 2-NA, 2-aminonaphthalene; Neq/24 h, urinary nicotine equivalents quantity excreted in 24 h; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides; 1-OHP, 1-hydroxypyrene and its glucuronide and sulfate conjugates; *S*-PMA, *S*-phenyl mercapturic acid.

[a] Groups abbreviated as: EHCSS-K6, EHCSS series-K heater and cigarette (5 mg tar, 0.3 mg nicotine, and 1.0 mg CO); CC, conventional cigarette.

[b] Baseline measures are mean values of Visits 2 and 3 and all subjects smoked conventional cigarettes.

[c] End-of-study values are mean values of Visits 7 and 8.

[d] Change from baseline to end of study.

[e] Statistically different from baseline ($p \leqslant 0.001$).

[f] Statistically different from baseline ($p \leqslant 0.01$).

[g] Statistically different from baseline ($p \leqslant 0.05$).

[h] Normal range in smokers is 0-5 ppm. No statistical analyses were performed for breath CO.

The biomarker 11-DTXB$_2$ is found to be increased up to 40% in smokers compared to non-smokers, and may be a prognostic indicator of CVD events (Calapai et al., 2009). It is a potent vasoconstrictor and an *in vivo* marker of platelet aggregation and activation, both of which may make it an indicator of atherosclerosis or a hypercoagulable state (Benowitz, 2003; Zedler et al., 2006). The dose-dependent platelet inhibition and anticoagulation effects of acetyl salicylic acid (ASA) in clinical settings, and the resultant protection against secondary CVD events, are thought to be explained by the effect of ASA on mechanisms involving thromboxane (EMEA, 2002). The changes observed regarding 11-DTXB$_2$ in the EHCSS-K6 group support the use of 11-DTXB$_2$ in future studies of tobacco products.

The reductions of biomarkers of exposure observed in this study for the EHCSS-K6 group were generally of a large magnitude for a broad spectrum of cigarette smoke HPHC and occurred despite the observed increase of the daily consumption of cigarettes. However, for many of the biomarkers the extent of the reductions were smaller in this study than in a previous EHCSS-K6 study performed in a confinement setting (with capped cigarette consumption) in the United Kingdom (Tricker et al., 2012a). For example COHb, *S*-PMA and nicotine equivalents were reduced by 70%, 80%, and 44% in the previous confinement study compared to 50%, 48% and 7%, respectively, in this study. This is noteworthy since a reduction of CC consumption and/or CC tar yields in many studies does not necessarily result in a reduction in biomarkers of exposure to HPHC (Hatsukami et al., 2006a,b; Lubin et al., 2007; Joseph et al., 2008).

While in short-term confinement studies, it is possible to obtain accurate data about the types and quantities of cigarettes smoked, longer-term ambulatory studies are reliant on accurate self-reporting. It would be beneficial to develop and validate a biomarker for detection of dual use of a test cigarette (e.g., EHCSS-K6) and CC.

It should be noted that the EHCSS-K6 cigarettes were supplied without charge in this study, whereas smokers of conventional cigarettes had to purchase their cigarettes. This is a potential source of bias for smoking behavior that may have contributed in the increased cigarette consumption in the EHCSS-K6 group.

5. Conclusions

In conclusion, the data from the current study indicate potential favorable changes in some biomarkers associated with CVD in smokers who have switched to the EHCSS-K6 cigarette over a 1-month period. Switching from conventional cigarettes to the EHCSS-K6 results in reductions in all biomarkers of exposure measured even with an increasing number of EHCSS-K6 cigarettes smoked, supporting previous findings. However, none of the reductions in biomarkers of exposure between the EHCSS-K6 and conventional cigarettes groups was significant. Future studies should investigate both larger sample sizes and longer durations of exclusive exposure to evaluate the effect on CVD biomarkers.

Conflict of interest statement

All authors are or were Philip Morris International (PMI) R&D employees or worked for PMI R&D under contractual agreements. The work reported in all eight parts of this supplement was funded by PMI R&D.

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References

Abel, G.A., Hays, J.T., Decker, P.A., Croghan, G.A., Kuter, D.J., Rigotti, N.A., 2005. Effects of biochemically confirmed smoking cessation on white blood cell count. Mayo Clin. Proc. 80, 1022–1028.

Andrews, J.O., Tingen, M.S., 2006. The effect of smoking, smoking cessation, and passive smoke exposure on common laboratory values in clinical settings: a review of the evidence. Crit. Care Nurs. Clin. North Am. 18, 63–69 (xii).

Bakhru, A., Erlinger, T.P., 2005. Smoking cessation and cardiovascular disease risk factors: results from the Third National Health and Nutrition Examination Survey. PLoS Med. 2, e160.

Bazzano, L.A., He, J., Muntner, P., Vupputuri, S., Whelton, P.K., 2003. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann. Intern. Med. 138, 891–897.

Benowitz, N.L., 2003. Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. Prog. Cardiovasc. Dis. 46, 91–111.

Benowitz, N.L., Jacob 3rd, P., Fong, I., Gupta, S., 1994. Nicotine metabolic profile in man: comparison of cigarette smoking and transdermal nicotine. J. Pharmacol. Exp. Ther. 268, 296–303.

Burns, D.M., 2003. Epidemiology of smoking-induced cardiovascular disease. Prog. Cardiovasc. Dis. 46, 11–29.

Calapai, G., Caputi, A.P., Mannucci, C., Russo, G.A., Gregg, E., Puntoni, R., Lowe, F., McEwan, M., Bassi, A., Morandi, S., Nunziata, A., 2009. Cardiovascular biomarkers in groups of established smokers after a decade of smoking. Basic Clin. Pharmacol. Toxicol. 104, 322–328.

Carmella, S.G., Han, S., Fristad, A., Yang, Y., Hecht, S.S., 2003. Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human urine. Cancer Epidemiol. Biomarkers Prev. 12, 1257–1261.

Deyton, L., Sharfstein, J., Hamburg, M., 2010. Tobacco product regulation: a public health approach. New Engl. J. Med. 362, 1753–1756.

Eliasson, B., Hjalmarson, A., Kruse, E., Landfeldt, B., Westin, A., 2001. Effect of smoking reduction and cessation on cardiovascular risk factors. Nicotine Tob. Res. 3, 249–255.

EMEA, 2002. EMEA/CPMP/EWP/282/02/Final-position paper on the regulatory requirements for the authorisation of low-dose modified release ASA formulations in the secondary prevention of cardiovascular events. <http://www.emea.europa.eu/pdfs/human/ewp/028202en.pdf>. (last updated 06.2002, accessed 05.2009).

Family Smoking Prevention and Tobacco Control Act (FSPTCA), 2009. Public Law No. 111–31 (22.06.2009).

Food and Drug Administration, 2001. Guidance for industry: Bioanalytical Method Validation. <http://www.fda.gov/cder/guidance/4252fnl.pdf>. (last updated 2001, accessed 04.23, 2009).

Food and Drug Administration, 2012. FDA's Guidance for Industry "Modified Risk Tobacco Products Applications". Request for Comments Docket No. FDA-2012-D-0071. Available at: <http://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/ucm297750.htm> (accessed 23.05.2012).

Frost-Pineda, K., Zedler, B.K., Liang, Q., Roethig, H.J., 2008a. Environmental tobacco smoke (ETS) evaluation of a third-generation electrically heated cigarette smoking system (EHCSS). Regul. Toxicol. Pharmacol. 52, 118–121.

Frost-Pineda, K., Zedler, B.K., Oliveri, D., Feng, S., Liang, Q., Roethig, H.J., 2008b. Short-term clinical exposure evaluation of a third-generation electrically heated cigarette smoking system (EHCSS) in adult smokers. Regul. Toxicol. Pharmacol. 52, 104–110.

Frost-Pineda, K., Zedler, B.K., Oliveri, D., Liang, Q., Feng, S., Roethig, H.J., 2008c. 12-Week clinical exposure evaluation of a third-generation electrically heated cigarette smoking system (EHCSS) in adult smokers. Regul. Toxicol. Pharmacol. 52, 111–117.

Hatsukami, D.K., Kotlyar, M., Allen, S., Jensen, J., Li, S., Le, C., Murphy, S., 2005. Effects of cigarette reduction on cardiovascular risk factors and subjective measures. Chest 128, 2528–2537.

Hatsukami, D.K., Benowitz, N.L., Rennard, S.I., Oncken, C., Hecht, S.S., 2006a. Biomarkers to assess the utility of potential reduced exposure tobacco products. Nicotine Tob. Res. 8, 600–622.

Hatsukami, D.K., Le, C.T., Zhang, Y., Joseph, A.M., Mooney, M.E., Carmella, S.G., Hecht, S.S., 2006b. Toxicant exposure in cigarette reducers versus light smokers. Cancer Epidemiol. Biomarkers Prev. 15, 2355–2358.

Heatherton, T.F., Kozlowski, L.T., Frecker, R.C., Fagerstrom, K.O., 1991. The Fagerström Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br. J. Addict. 86, 1119–1127.

Hecht, S.S., 2003. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat. Rev. Cancer 3, 733–744.

Institute of Medicine, 2012. Scientific Standards for Studies on Modified Risk Tobacco Products. The National Academy Press, Washington, DC.

International Conference on Harmonisation (ICH), 1996. International Conference on Harmonisation. ICH Tripartite Guidelines for Good Clinical Practice. Practice Note for Guidance on Good Clinical Practice (ICH Topic E6, Step 5). Adopted by CPMP, issued as CPMP/ICH/135/95.

International Organization for Standardization, 1991. Tobacco and Tobacco Products – Atmosphere for Conditioning and Testing. ISO 3402.. International Organization for Standardization, Geneva.

International Organization for Standardization, 1995. Cigarettes – Determination of Carbon Monoxide in the Vapour Phase of Cigarette Smoke – NDIR Method. ISO 8454, second ed. International Organization for Standardization, Geneva.

International Organization for Standardization, 2000a. Routine Analytical Cigarette-Smoking Machine – Definitions and Standard Conditions. Standard 3308, fourth ed. International Organization for Standardization, Geneva.

International Organization for Standardization, 2000b. Cigarettes – Determination of Total and Nicotine-Free Dry Particulate Matter Using a Routine Analytical Smoking Machine. ISO Standard 4387, third ed. International Organization of Standardization, Geneva.

International Organization for Standardization, 2000c. Cigarettes – Determination of Nicotine in Smoke Condensates – Gas Chromatographic Method. ISO Standard 10315, second ed. International Organization for Standardization, Geneva.

Joseph, A.M., Hecht, S.S., Murphy, S.E., Lando, H., Carmella, S.G., Gross, M., Bliss, R., Le, C.T., Hatsukami, D.K., 2008. Smoking reduction fails to improve clinical and biological markers of cardiac disease: a randomized controlled trial. Nicotine Tob. Res. 10, 471–481.

Kannel, W.B., Anderson, K., Wilson, P.W., 1992. White blood cell count and cardiovascular disease. Insights from the Framingham study. JAMA 267, 1253–1256.

Kapur, N.K., Ashen, D., Blumenthal, R.S., 2008. High density lipoprotein cholesterol: an evolving target of therapy in the management of cardiovascular disease. Vasc. Health Risk Manag. 4, 39–57.

Lavi, S., Prasad, A., Yang, E.H., Mathew, V., Simari, R.D., Rihal, C.S., Lerman, L.O., Lerman, A., 2007. Smoking is associated with epicardial coronary endothelial dysfunction and elevated white blood cell count in patients with chest pain and early coronary artery disease. Circulation 115, 2621–2627.

Lindner, D., Smith, S., Martin Leroy, C., Tricker, A.R., 2011. Comparison of exposure to selected cigarette smoke constituents in adult smokers and nonsmokers in a European, multicenter, observational study. Cancer Epidemiol. Biomarkers Prev. 20, 1524–1536.

Lubin, J.H., Caporasso, N., Hatsukami, D.K., Joseph, A.M., Hecht, S.S., 2007. The association of a tobacco-specific biomarker and cigarette consumption and its dependence on host characteristics. Cancer Epidemiol. Biomarkers Prev. 16, 1852–1857.

Mascher, D.G., Mascher, H.J., Scherer, G., Schmid, E.R., 2001. High-performance liquid chromatographic-tandem mass spectrometric determination of 3-hydroxypropylmercapturic acid in human urine. J. Chromatogr. B: Biomed. Sci. Appl. 750, 163–169.

Medeiros, A.M., Bird, M.G., Witz, G., 1997. Potential biomarkers of benzene exposure. J. Toxicol. Environ. Health 51, 519–539.

Minami, J., Todoroki, M., Yoshii, M., Mita, S., Nishikimi, T., Ishimitsu, T., Matsuoka, H., 2002. Effects of smoking cessation or alcohol restriction on metabolic and fibrinolytic variables in Japanese men. Clin. Sci. (Lond.) 103, 117–122.

Perkins, K.A., Karelitz, J.L., Giedgowd, G.E., Conklin, C.A., 2012. The reliability of puff topography and subjective responses during ad lib smoking of a single cigarette. Nicotine Tob. Res. 14, 490–494.

Pojer, R., Whitfield, J.B., Poulos, V., Eckhard, I.F., Richmond, R., Hensley, W.J., 1984. Carboxyhemoglobin, cotinine, and thiocyanate assay compared for distinguishing smokers from non-smokers. Clin. Chem. 30, 1377–1380.

Riedel, K., Scherer, G., Engl, J., Hagedorn, H.W., Tricker, A.R., 2006. Determination of three carcinogenic aromatic amines in urine of smokers and nonsmokers. J. Anal. Toxicol. 30, 187–195.

Roethig, H.J., Kinser, R.D., Lau, R.W., Walk, R.A., Wang, N., 2005. Short-term exposure evaluation of adult smokers switching from conventional to first-generation electrically heated cigarettes during controlled smoking. J. Clin. Pharmacol. 45, 133–145.

Roethig, H.J., Zedler, B.K., Kinser, R.D., Feng, S., Nelson, B.L., Liang, Q., 2007. Short-term clinical exposure evaluation of a second-generation electrically heated cigarette smoking system. J. Clin. Pharmacol. 47, 518–530.

Roethig, H.J., Feng, S., Liang, Q., Liu, J., Rees, W.A., Zedler, B.K., 2008. A 12-month, randomized, controlled study to evaluate exposure and cardiovascular risk factors in adult smokers switching from conventional cigarettes to a second-generation electrically heated cigarette smoking system. J. Clin. Pharmacol. 48, 580–591.

Roethig, H.J., Koval, T., Muhammad-Kah, R., Jin, Y., Mendes, P., Unverdorben, M., 2010. Short term effects of reduced exposure to cigarette smoke on white blood cells, platelets and red blood cells in adult cigarette smokers. Regul. Toxicol. Pharmacol. 57, 333–337.

Schorp, M.K., Tricker, A.R., Dempsey, R., 2012. Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 1: non-clinical and clinical insights. Regul. Toxicol. Pharmacol. 64 (Suppl. 1), S1–S10.

Strickland, P., Kang, D., Sithisarankul, P., 1996. Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. Environ. Health Perspect. 104 (Suppl. 5), 927–932.

Tricker, A.R., Schorp, M.K., Urban, H.J., Leyden, D., Hagedorn, H.W., Engl, J., Urban, M., Riedel, K., Gilch, G., Janket, D., Scherer, G., 2009. Comparison of environmental tobacco smoke (ETS) concentrations generated by an electrically heated cigarette smoking system and a conventional cigarette. Inhal. Toxicol. 21, 62–77.

Tricker, A.R., Stewart, A.J., Martin Leroy, C., Lindner, D., Schorp, M.K., Dempsey, R., 2012a. Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 3: 8-day randomized clinical trial in the UK. Regul. Toxicol. Pharmacol. 64 (Suppl. 1), S35–S44.

Tricker, A.R., Jang, I.-J., Martin Leroy, C., Lindner, D., Dempsey, R., 2012b. Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 4: 8-day randomized clinical trial in Korea. Regul. Toxicol. Pharmacol. 64 (Suppl. 1), S45–S53.

Tricker, A.R., Kanada, S., Takada, K., Martin Leroy, C., Lindner, D., Schorp, M.K., Dempsey, R., 2012c. Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 5: 8-day randomized clinical trial in Japan. Regul. Toxicol. Pharmacol. 64 (Suppl. 1), S54–S63.

Tricker, A.R., Lindner, D., Martin Leroy, C., Schorp, M.K., Dempsey, R., 2012d. Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 6: 6-day randomized clinical trial of a menthol cigarette in Japan. Regul. Toxicol. Pharmacol. 64 (Suppl. 1), S64–S73.

US Department of Health and Human Services, 2010. A Report of the Surgeon General: How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-attributable Disease. Department of Health and Human Services, Public Health Services, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, Rockville, MD.

Uppsala Monitoring Center, 2003. WHO Drug Dictionary Enhanced. Uppsala, Sweden. Available at: <http://www.umc-products.com/DynPage.aspx?id=73588&mn1=1107&mn2=1139> (quarter 4).

van Sittert, N.J., Megens, H.J., Watson, W.P., Boogaard, P.J., 2000. Biomarkers of exposure to 1,3-butadiene as a basis for cancer risk assessment. Toxicol. Sci. 56, 189–202.

Werley, M.S., Freelin, S.A., Wrenn, S.E., Gerstenberg, B., Roemer, E., Schramke, H., Van Miert, E., Vanscheeuwijck, P., Weber, S., Coggins, C.R.E., 2008. Smoke chemistry, in vitro and in vivo toxicology evaluations of the electrically heated cigarette smoking system series K. Regul. Toxicol. Pharmacol. 52, 122–139.

World Medical Association (WMA), 1964 and regularly amended. World Medical Association. Declaration of Helsinki-recommendations guiding physicians in biomedical research involving human subjects.

Zedler, B.K., Kinser, R., Oey, J., Nelson, B., Roethig, H.J., Walk, R.A., Kuhl, P., Rustemeier, K., Schepers, G., Von Holt, K., Tricker, A.R., 2006. Biomarkers of exposure and potential harm in adult smokers of 3–7 mg tar yield (Federal Trade Commission) cigarettes and in adult non-smokers. Biomarkers 11, 201–220.

Zenzen, V., Diekmann, J., Gerstenberg, B., Weber, S., Wittke, S., Schorp, M.K., 2012. Reduced exposure evaluation of an Electrically Heated Cigarette Smoking System. Part 2: Smoke chemistry and in vitro toxicological evaluation using smoking regimens reflecting human puffing behavior. Regul. Toxicol. Pharmacol. 64 (Suppl. 1), S11–S34.